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Plasmodium vivax (Grassi and Feletti, 1890)

SYNONYMS: *Haemamoeba malariae* Feletti and Grassi, 1890, *partim; Haemamoeba vivax* Grassi and Feletti, 1890; *Plasmodium malariae tertianae* Celli and Sanfelice, 1891; *Plasmodium malariae tertianae* Kruse, 1892; *Haemamoeba laverani* var. *tertiana* Labbe, 1894 (?) ; *Haemosporidium tertianae* Lewkowicz, 1897; *Haemamoeba malariae tertianae* Laveran, 1901; *Plasmodium camarense* Ziemann, 1915.

The first person to see and describe the true malaria parasite of man was the French army surgeon, Louis Alphonse Laveran. There is little doubt that during his investigations of 1880 and 1881 he saw the human tertian parasite in the blood of patients at Contantine, Algeria. He made no attempt to attach names other than calling the organisms *Oscillaria malariae* referring, undoubtedly, to the fine hair-like projections he saw develop from a pigmented spherical body in fresh blood from a patient with malaria.

The parasite of tertian malaria was well recognized by Golgi (1886, 1889). In the first paper, he mentioned that a tertian parasite must have a developmental cycle different from quartan. He then proceeded to describe the course of the disease during four tertian attacks (giving Prof. Reva credit for allowing him to use his patient), including the morphology of the parasites in relation to the paroxysm. In the more extensive paper of 1889, he re-affirmed his earlier findings and presented figures of the development of the parasite in the red cell with amazing accuracy. Even though he clearly separated tertian from quartan malaria, he did not name the parasite of either one. In an addendum to a paper on the malaria of birds, Grassi and Feletti, 1890, gave the name *vivax* for the human tertian parasite under the genus *Haemamoeba.* In 1885, Marchiafava and Celli had proposed *plasmodium* as the genus name for the malaria parasites with the result that the combination *Plasmodium vivax* (Grassi and Feletti, 1890) came into general use but lacked official status until the International Commission on Zoological Nomenclature, after much soul searching, made the historic decision, opinion 283, to validate the names of the human malaria parasites in common use (see Hemming, 1954).

Vivax malaria has a worldwide distribution but makes its greatest inroads in temperate climates. The disease is more or less confined to the lowlands, coastal areas, marshes, lake margins, and reclaimed sea beds. There are no known strains of the parasite which can complete their sporogonous development at temperatures below 15° C (59°F) and consequently vivax is stopped north and south of the equator, by summer isotherms of 15° or 16° C.

In the tropics, *P. vivax* may predominate over *P. falciparum,* as in the lower Amazon, but the absolute prevalence in any area may not be known because of the rapid build-up of immunity to all strains of the parasite, in areas of high transmission, with consequent suppression of parasitemia. This situation was well demonstrated by Missoroli (1932). The highest incidence under present conditions is probably in Asia where it extends as a wide belt across the entire continent.

In 1949, Brumpt mentioned what he called the "benign tertian mystery" in writing about the situation in Liberia, Gabon, Lagos, and Stanleyville where, in the presence of efficient vectors of *P. vivax,* no vivax malaria occurs.

Garnham (1966) enlarged upon this by including "most of West Africa in the belt between the Congo and Mauritania." The explanation may be that the indigenous population is highly refractory to this parasite. This phenomenon was ably investigated by Young *et al* (1955) who studied many different strains of *P. vivax* during some 20 years, and found that only 23 percent of American Negroes came down with developed infections as against 96 percent of Caucasians; even when massive numbers of parasites were introduced, the susceptibility rate remained the same.

The rule of thumb is that pronounced resistance to *P. vivax* is confined to the true Negro but like many such rules, there are exceptions, as the senior author learned when he encountered a very sick true Negro in the malaria ward at Fort Benning, Ga. in 1951. The record showed that the patient was infected with *P. vivax* acquired in Korea. This was questioned with some impatience; whereupon, a fresh smear was made, stained, and examined by the senior author. *It was P. vivax!* This was one of many American Negroes who acquired vivax malaria in Korea and in whom the natural history and clinical attacks appeared no different than the same infections in Caucasians (Hankey *et al,* 1953). It is interesting that on the same day the above episode was enacted at Fort Benning, the late Dr. Alf Alving had the exact same experience at Fort Dix, N.J.

Beginning in 1900, many investigators have studied different strains of *P. vivax* and among these, the ones which received the most attention were the Madagascar strain (James, 1931 and James *et al,* 1936), the Dutch strain (Schüffner *et al,* 1929), the McCoy strain (Boyd, 1940; Boyd and Kitchen, 1944), and the New Guinea strain (Fairley *et al,* 1945). In our own work, we have undertaken studies on many different strains from various parts of the world, but have concentrated our efforts on the St. Elizabeth (see Coatney and Young, 1941; Coatney and Cooper, 1948; Coatney *et al,* 1950a), and the Chesson strain (Ehrman *et al,* 1945; Coatney and Cooper, 1948). The last two, along with certain others, will be discussed more fully later in this chapter.

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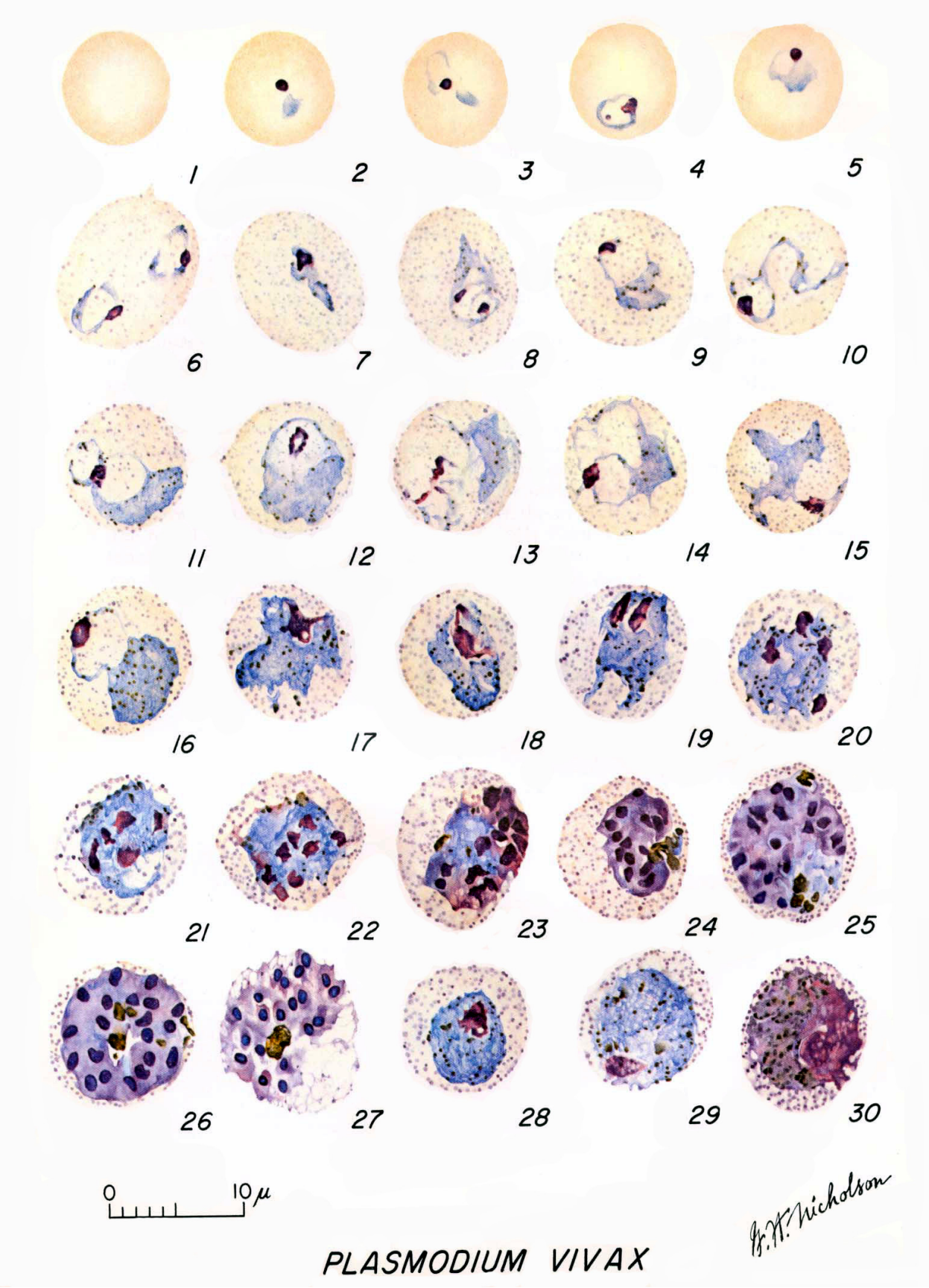
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PLASMODIUM VIVAX
G. H. Nicholson

Cycle in the Blood
PLATE I

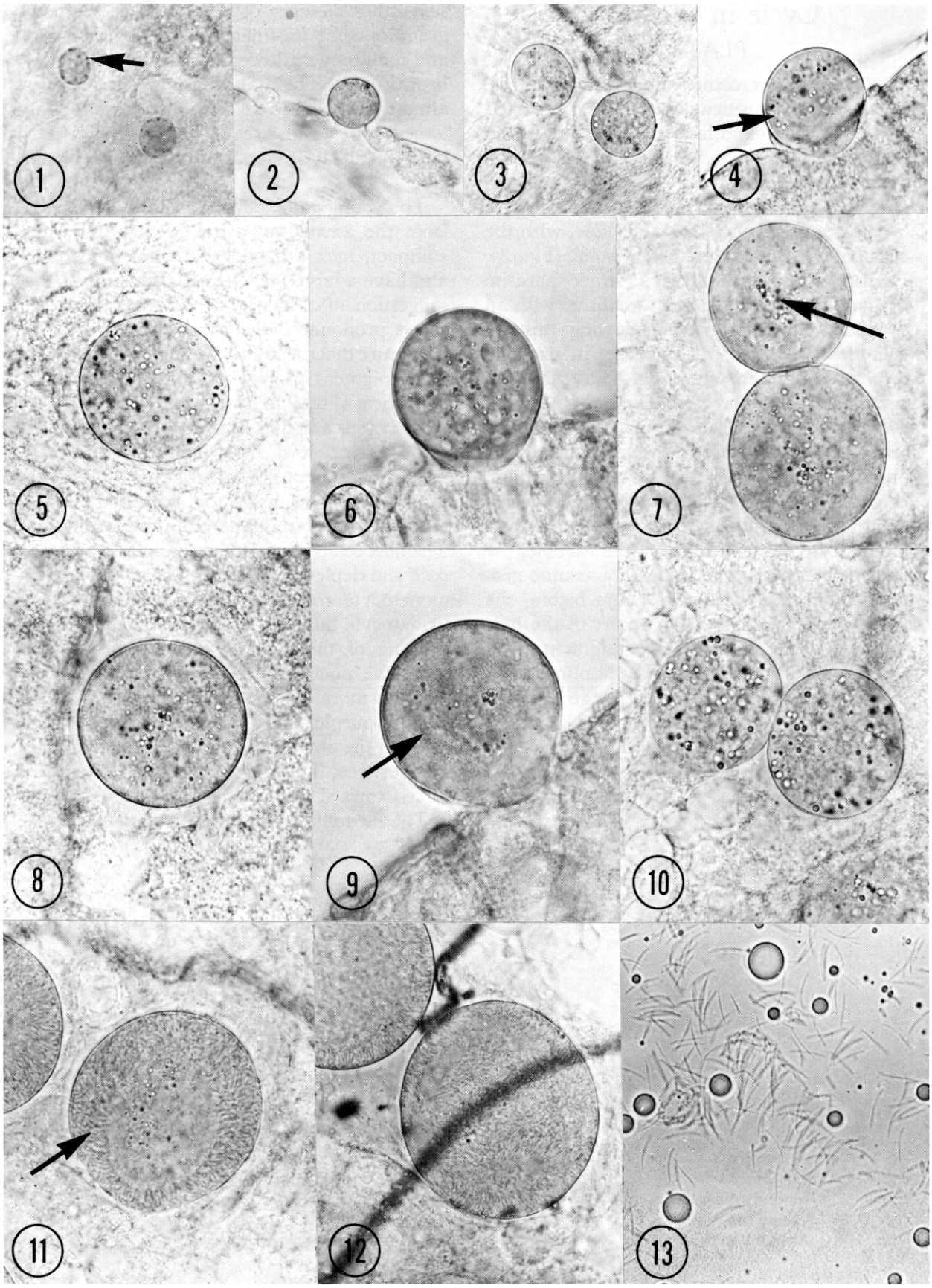
The young merozoite enters the red blood cell, generally a reticulocyte, as first reported by Craik (1920) and later verified by Kitchen (1938) and others, where it appears with a deep red nucleus and a whisp of cytoplasm (Fig. 2). As the parasite grows, a loop of cytoplasm forms enclosing a vacuole, with the circular nucleus at the anchor point (Fig. 3). Further growth produces a larger parasite with a distinct vacuole and sometimes with an accessory chromatin dot. Following this development, the red cell increases in size and displays Schüffner's stippling. The cytoplasm of the parasite is increased in amount, becomes decidedly amoeboid, and exhibits very small granules of light brown pigment. Multiple invasion of the host red cell is not uncommon (Figs. 4-10). The remainder of the development of the trophozoite consists of concentrating the cytoplasm with loss of the vacuole, the nucleus becomes larger and may assume grotesque shapes. Where only hours before, the trophozoite occupied a large part of the host cell, it now becomes compact, pigment granules become prominent, and stippling more intense (Figs. 11-18). The first stage of schizogony results in 2 nuclei and, then, in rather rapid succession, other divisions occur, to deliver up to 24 nuclei; the usual number is 16, although certain strains have 18 or more consistently. With the process of nuclear division other changes occur, too. The pigment granules come together to form larger chunks and then they coalesce into a single yellowish-brown lump. The maturing schizont occupies almost the whole red cell with the stippling forced into a rim-area. The host cell is enlarged and its cytoplasm appears depleted (Figs. 19-27).

The young gametocytes are easily separated from the asexual parasites because they are compact, lack a vacuole, are not amoeboid, and have a large nucleus with, sometimes, the suggestion of a halo. The enlarged host cell shows pronounced Schüffner's stippling. The immature macrogametocyte stains a deep blue. Dark pigment granules are scattered in the cytoplasm (Fig. 28). The mature macrogametocyte stains a lighter blue than the developing form and occupies most of the host cell. Its nucleus is generally eccentric and may show a darker portion inside the main body. Dark pigment grains are scattered in the cytoplasm. The host cell is enlarged, its cytoplasm appears pale and depleted (Fig. 29). The young microgametocyte resembles the immature macrogametocyte but as it approaches maturity, the staining of the cytoplasm is toward bluish-gray, the nucleus is larger, occupying about half or more of the parasite, and takes a reddish-purple stain. The dark pigment is confined to the area of the cytoplasm, leaving the nucleus free. The stippling of the host cell is forced toward its periphery (Fig. 30).

The asexual cycle takes 48 hours.

PLATE I. – *Plasmodium vivax*

Fig. 1. Normal red cell.
Fig. 2-5. Young trophozoites.
Fig. 6-16. Growing trophozoites.
Figs. 17, 18. Mature trophozoites.
Figs. 19-21. Early schizonts.
Figs. 22, 23. Developing schizonts.
Figs. 24-27. Nearly mature and mature schizonts.
Figs. 28-29. Nearly mature and mature macrogametocytes.
Fig.30. Mature microgametocyte.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Sporogonic Cycle
PLATE II

Bano (1959) observed two small dot-like and two rod-like chromosomes in 49-hour oocysts of *P. vivax* in *Anopheles stephensi* mosquitoes. The haploid number of chromosomes was determined to be two. The size of oocysts at this time ranged from 11 to 14 μ in diameter. Although many workers have observed the oocysts of *P. vivax,* the studies of Shute and Maryon (1952) more nearly approximate our observations. These workers observed the development of oocysts of this parasite, at a temperature of 25°C, in *A. atroparvus* mosquitoes. They reported that the 50 to 100 greenish-brown pigment granules did not form a pattern in the very young oocysts. By days 4 and 5, however, there was a tendency toward chain formation, often in 3 lines. By day 6, the pigment was practically obscured and by day 7, only a few grains were observable. The oocysts were 10 to 46 μ in diameter. From day 3 to 6, the daily increase in diameter was about 7 μ; between the 6th and 7th day, however, growth became more rapid. Sporogony was completed in 9 days.

In our studies, observations were made on the oocyst development of the Chesson strain of *P. vivax,* in *A. b. balabacensis A. freeborni, A. maculatus, A. stephensi,* and *A. quadrimaculatus* mosquitoes incubated at a temperature of 25° C (Table 1).

In *A. b. balabacensis,* the oocysts, at day 4, ranged from 9 to 15 μ in diameter with a mean of 12 μ. The oocysts continued to grow so that by day 11, the size ranged from 18 to 59 μ with a mean of 46 μ. Sporozoites were present in the salivary glands by day 12. In *A. freeborni,* the mean oocyst diameters were generally greater than seen in the *A. b. balabacensis;* at day 11, the size of the oocysts ranged from 25 to 67 μ with a mean of 46 μ; sporozoites were present in the salivary glands. The oocyst diameters in the other species of mosquitoes fell within the range of these 2 mosquitoes. Sporozoites were present in the salivary glands of *A. maculatus* and *A. stephensi* by day 11 and in the salivary glands of *A. quadrimaculatus* by day 12.

In this, and in subsequent chapters, the growth of the oocysts is compared with those of *P. cynomolgi.* In each instance, the comparison is made from measurements made on the same species of mosquito. In this case, the comparison is between the growth curves of *P. vivax* and *P. cynomolgi* in *A. freeborni* mosquitoes (Fig. 6). *Plasmodium cynomolgi* has a larger mean oocyst diameter than *P. vivax* and much larger maximum oocyst diameter. Both of the parasites completed their development, as measured by the presence of sporozoites in the salivary glands, by day 11.

PLATE II

FIGURES 1 – 13. – Developing oocysts and sporozoites of *Plasmodium vivax* in *Anopheles maculates, A. freeborni, A. quadrimaculatus*, and *A. b. balabacensis* mosquitoes. X 580.

Fig. 1. 4-day oocysts showing peripheral pigment.
Fig. 2. 5-day oocyst
Fig. 3. 6-day oocysts.
Fig. 4. 7-day oocyst showing pigment and small vacuoles.
Fig. 5. 8-day oocyst.
Fig. 6. 9-day oocyst.
Fig. 7. 9-day oocysts showing concentration of vacuoles.
Fig. 8. 10-day oocyst.
Fig. 9. 10-day oocyst showing early formation of sporoblasts.
Fig. 10. 11-day oocysts showing prominent vacuolation.
Fig. 11. 12-day oocyst showing differentiation.
Fig. 12. Fully differentiated 12-day oocyst.
Fig. 13. Sporozoites present near salivary gland tissue 12 days after feeding.

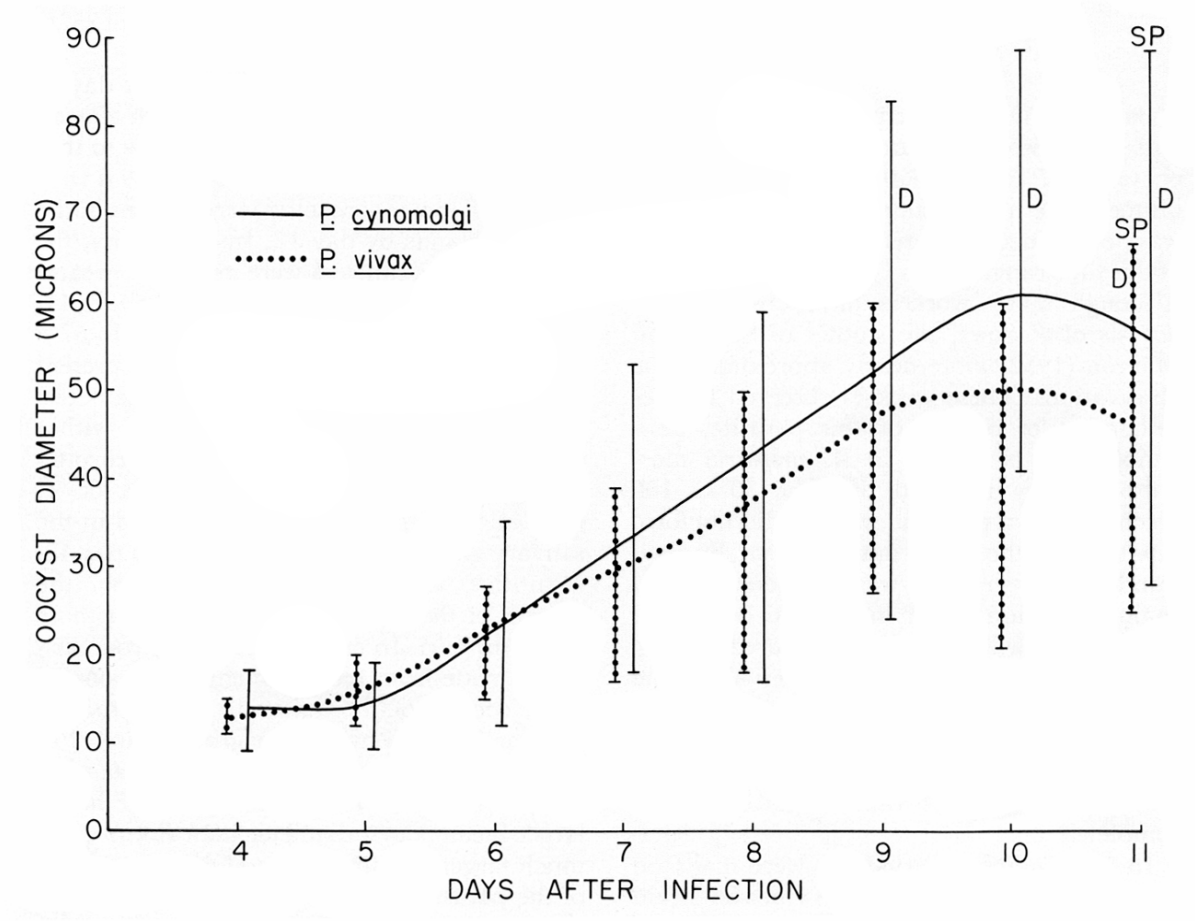

FIGURE 6.—A comparison of the mean oocyst diameter curve and ranges in oocyst diameters of *Plasmodium vivax* and *P. cynomolgi* in *Anopheles freeborni* mosquitoes. (D = oocyst differentiation; SP = sporozoites present in the salivary glands).

TABLE 1.-- Oocyst diameters of *Plasmodium vivax* in *Anopheles b. balabacensis, A. freeborni, A. maculatus, A. stephensi,* and *A. quadrimaculatus* mosquitoes.

Days after Infection	*A. b. balabacensis*			*A. freeborni*			*A. maculatus*			*A. stephensi*			*A. quadrimaculatus*		
	No.	Range	Mean *	No.	Range	Mean *	No.	Range	Mean *	No.	Range	Mean *	No.	Range	Mean *
4	100	9-15	12	100	11-15	13	100	11-17	13	56	11-15	13	102	11-14	12
5	100	12-19	16	76	12-20	16	53	11-19	15	100	12-18	14	36	12-18	15
6	100	12-26	22	92	15-28	23	17	18-26	22	100	14-27	22	64	13-25	22
7	100	13-32	25	100	17-39	30	100	15-35	27	100	18-42	31	100	18-31	25
8	100	18-40	32	100	18-50	36	100	‡	34	100	24-51	40	100	21-48	36
9	100	18-42	31	100	27-60	48	100	‡	40	100	30-59	14	100	18-52	41
10	100	18-61	45	100	21-60	50	100	‡	47	100	31-67	53	100	24-59	45
11	100	18-59	46†	95	25-67	46†**	100	26-66	47†**	100	24-65	48†**	100	21-59	49†
12			†**			†**							100	27-59	47†**
Totals	800	9-61		763	11-67		670	11-66		756	11-67		802	11-59	

* Measurements expressed in microns.
† Oocyst differentiation.
** Sporozoites present in the salivary glands.
‡ Ranges in oocyst diameters not available.

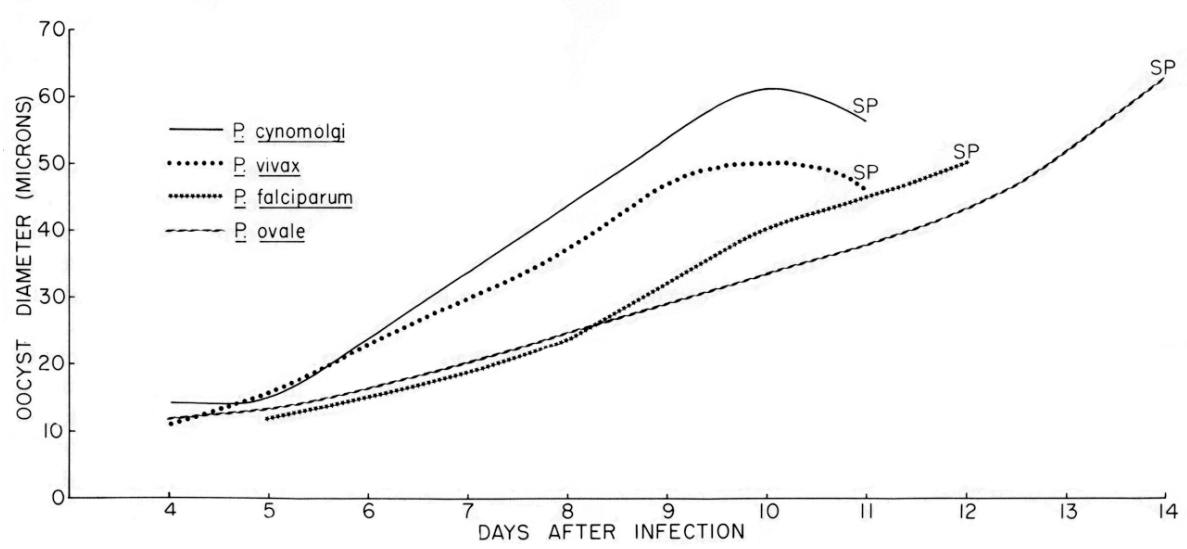

FIGURE 7—A comparison of the mean oocyst diameter curves of *Plasmodium cynomolgi, P. vivax, P. falciparum, and P. ovale* in *Anopheles freeborni* mosquitoes.

A comparison of the oocyst growth curves of the 3 human tertian malarias *(P. vivax, P. falciparum,* and *P. ovale)* with *P. cynomolgi* (Fig. 7) shows several distinct differences. Although *P. vivax* has a smaller mean diameter than *P. cynomolgi,* the shape of its growth curve is strikingly similar to that of *P. cynomolgi.* In contrast, *P. falciparum* and *P. ovale* have more of a straight line growth curve. Whereas sporozoites are present in the salivary glands of mosquitoes infected with *P. vivax* and *P. cynomolgi* by day 11, *P. falciparum* and *P. ovale* require 12 and 14 days, respectively for their development.

The sporozoites are narrow, elongate bodies, either straight or slightly curved, with one end more blunted than the other. The length of the living sporozoite is 14 μ and in dried preparations, is 1 to 2 μ less (Garnham, 1966). Using electron microscopy, Garnham *et al* (1963) gave detailed descriptions of the structures present in this form of the parasite which displays a complicated morphology.

We have readily transmitted the infection to man via the bites of *A. freeborni, A. quadrimaculatus, A. b. balabacensis,* and *A. maculatus* mosquitoes.

Cycle in the Tissue

The first tissue stages of a human malaria parasite, *P. vivax,* were demonstrated by Shortt and Garnham (1948). These tissue stages were found in a 7-day liver biopsy taken from a human volunteer upon whom approximately 1,728 anopheline mosquitoes, infected with *P. vivax,* were allowed to bite on 2 successive days. In addition, 200 pairs of salivary glands were dissected out from the same mosquitoes, and inoculated intravenously. Schizonts, considered to be 6- and 7-day forms, were described from the liver tissue taken at biopsy. The 6-day forms were ovoid masses similar to those seen in *P. cynomolgi* except that they were larger, about 42 μ in their greatest dimension. The various shapes, the vacuolation, the absence of tissue reaction, and the staining characteristics of the cytoplasm and chromatin were also similar to what they had first observed in *P. cynomolgi.* The 7-day stage, reported by these authors, consisted of a single, fully or nearly mature form, since merozoites were seen escaping from it.

The youngest *P. vivax* tissue stage was described by Rodhain in 1956 in liver biopsy material from a chimpanzee which had been inoculated with sporozoites of vivax malaria 7 and 4 days earlier. These 4-day forms (possibly 7-day tissue stages) ranged in size from 24 μ in diameter up to 47.7 by 35.2 μ. The nuclei, or chromatin masses, measured slightly over 2 μ and were rather sparsely distributed. Rodhain considered these forms to be similar to those described for

man by Shortt and Garnham (1948).

Bray (1957) described 8- and 15-day tissue stages seen in liver biopsy material from a chimpanzee inoculated with sporozoites of *P. vivax* from *A. gambiae* mosquitoes. The 8-day *P. vivax* schizonts were similar to, but larger than, the 7-day forms demonstrated by Shortt and Garnham in 1948. He described two distinct stages of immature schizonts. The EE bodies averaged 52 by 44 μ and were almost always oval in shape. The immature stage contained 1 to 2 large vacuoles, cytoplasm was abundant and, at times, collected into darker staining aggregates. Occasionally, many small vacuoles were present, arranged around most of the periphery. Nuclei were relatively sparse. No clefts were observed. Bray also described premature and mature schizonts. In the premature stage, nuclei had completed their final division but with little or no change in their shape or size; the vacuoles had disappeared. The nuclei are elongate or form bars or loops; the cytoplasm breaks up and condenses upon the nuclei. This process of merozoite formation is completed in a very short time. The mature schizont is ovoid without any appreciable increase in size. They are packed with spherical merozoites which measure 1 to 1.4 μ in diameter. When the mature schizont ruptures, the merozoites are released. The size of the free merozoite is 0.8 to 1.2 μ.

Bray observed no patterns of nuclear arrangement or septal formation of the cytoplasm in any of the 8-day tissue stages of vivax malaria. There was no tissue reaction surrounding the EE bodies and no hypertrophic changes in the host-cell (hepatic) nuclei.

The tissue stages found in chimpanzee liver by Bray 15 days after exposure to infection appeared similar to the 8-day schizonts. There were no features which allowed for distinguishing these EE bodies as second generation forms. Secondary exoerythrocytic tissue stages were also found and described from a chimpanzee 9 months after inoculation with vivax sporozoites by Rodhain (1956a). Three of the 4 late tissue stages were elongate, irregular, or regular shaped ovals measuring 81, 61, and 49 μ in greatest dimension while the fourth form was lobulated.

The concensus is that there is a decided diminution in the number of tissue stages per measure of liver as the infection continues. Bray demonstrated this in his study of *P. vivax* in chimpanzees. With the inoculum used by him, Bray estimated a decrease from 20,000 primary exoerythrocytic forms (8-day biopsy) per mm^3 of liver to 500 secondary exoerythrocytic forms in the 15-day biopsy material.

There can be no question that the liver cycle of vivax malaria persists for upwards of several years since many strains of vivax continue to exhibit true relapses through a minimum of 2 to 3 years. Ciuca *et al* (1955) observed relapses up to 8 years. It is doubtful that these latter were true relapses. Professor Gh. Lupascu, one of the late Dr. Ciuca's colleagues, has recently written one of us (PGC) that there was a good possibility of reinfection in their patients. Many of them had been released from the hospital for varying intervals and could easily have been reexposed to infection because malaria was at that time endemic to the area.

Before we leave the discussion of the *P. vivax* cycle in the fixed tissue, mention should be made of Fairley's monumental work (1947) on the observations of various phases of the vivax life cycle. In this work, he subinoculated large volumes of blood (200 ml.) from individuals heavily exposed to infection with vivax malaria by mosquito bites. He observed that subinoculation during the first 30 minutes after exposure to infection resulted in eventual patent infection in the subinoculee. After 30 minutes and through 7 days, subinoculations were non-infective. On the 8th day subinoculation was infective, indicating that the tissue schizonts had liberated merozoites into the circulating blood on day 8.

Course of Infection

Fairley's experiments (1947) showed that the earliest prepatent period (i.e., the time from infection, day 0, to the time parasites become microscopically detectable in the circulating blood) could be as early as 8 days; this has been observed, but only rarely. In the last 10 years, we have encountered only one prepatent period of 8 days (Coatney *et al,* 1963).

In addition, Boyd and Kitchen (1937) and Ciuca *et al* (1937) reported one 8-day prepatent period each in a large series of patients whose infections were induced by bites of infected mosquitoes. Putnam *et al* (1947) reported a 7-day prepatent period. However, this would be an 8-day period according to the manner in which all other investigators calculate the prepatent period. Kitchen (1949) reported 5 prepatent periods of 8 days in work with 9 different strains of vivax.

Generally, the duration of the prepatent period reported for many strains of *Plasmodium vivax* (Chesson, St. Elizabeth, Madagascar, McCoy, New Guinea, Roumanian, South Vietnam, Scanlon, West Pakistan, and Venezuelan) have ranged from 8 to 27 days, with medians or means ranging from 10.5 to 19 days (Boyd and Kitchen, 1937; Ciuca *et al,* 1937; Fairley *et al,* 1947; Coatney *et al,* 1950; Coatney *et al,* 1950a; Contacos and Coatney, 1963; Winckel, 1955).

Other strains of vivax malaria exhibit delayed prepatent periods, sometimes called protracted incubation periods, but probably more accurately described as delayed primaries.

The St. Elizabeth strain of vivax malaria (Coatney *et al,* 1950) is usually characterized by a short prepatent period of 11 to 18 days after infection. Only 3 volunteers out of 123 exhibited delayed primary attacks at 298 to 319 days after exposure to infection. Coatney and Cooper (1948) reviewed and described the characteristic of bimodal activity (parasitic and/or clinical) of a large number of vivax strains. They related the transmission studies of Sir Patrick Manson (1900) who exposed his son, P. Thurburn Manson, to infection with vivax malaria by bites of mosquitoes sent from Italy. A primary attack of malaria was experienced after 2 weeks and the attack was treated with quinine. Some 9 months later, a relapse of vivax malaria was experienced by Sir Patrick's son. There then followed many reports relating to 8- to 9-month intervals between primary and relapse activity, fitting more or less into a pattern for many strains of vivax in many countries. Hackett (1937) pointed out how this characteristic provided an explanation for spring malaria and for the overwintering of the parasite, and suggested, that strains of this type would have greatly enhanced chances for survival in temperate zones.

Yorke (1925) observed relapses in patients 6 to 13 months after exposure to infection. James (1931) and James *et al* (1936) observed late relapses with the Madagascar strain of vivax malaria. Schüffner *et al* (1929) observed prolonged "latency" in the form of delayed primary attacks in 8 of 8 patients exposed to infection with the Netherlands strain of vivax malaria. Boyd and Kitchen (1944) and Shannon *et al* (1948) observed the bimodal activity pattern (late relapses) with experimentally induced infections with the McCoy (U.S.) strain of vivax malaria. The Korean strain also exhibited the bimodal pattern of clinical activity and a period of long-term latency (Hankey *et al,* 1953).

Proof that such a consistently long period of time obtained between primary and relapse activity is common was furnished by studies of Coatney *et al* (1950) in volunteers who showed delayed relapse activity approximately 9 months after exposure to infection. They showed also that the time characteristic was independent of the season by exposing volunteers to infection during 9 months of the year. Relapses appeared only as a function of time with median periods of latency between 194 and 300 days.

Coatney and Cooper (1948) stated that morbidity statistics during World War II clearly showed that the vivax malarias from the Solomon Islands, New Guinea, and other areas of the Southwest Pacific did not exhibit consistent patterns of prolonged latency in their relapse mechanisms. Fairley (1945), working with vivax strains from New Guinea, reported no patterns of delayed relapse.

Coatney and Cooper (1948) summarized their studies with 2 strains of vivax malaria, used in drug evaluation studies in prisoner volunteers, the St. Elizabeth (U.S.A.) and the Chesson (New Guinea-South Pacific). The activity pattern of the St. Elizabeth strain is consistent with a relatively short prepatent period, between 7 and 14 days. This is followed by a period of many months when fixed-tissue stages remain quiescent, and then, by a late period of activity, with repeated

spaced invasions of red blood cells which may continue for upwards of 2 years, sometimes, longer.

The Chesson strain pattern of activity is radically different from that of the St. Elizabeth strain. The former is characterized by fairly regular reinvasions of the blood stream, after the original attack, from fixed-tissue stages, gradually becoming more widely spaced. In heavy infections, relapses continue for upwards of 18 months, occasionally, longer.

The authors in commenting on the differences between the Chesson and the St. Elizabeth strains stated that the differences in relapse activity between the 2 strains was in keeping with the presumed tropical origin of the Chesson and the temperate zone origin of the St. Elizabeth strain.

Winckel (1955) presented material which appears to be somewhat paradoxical; namely, that the Netherlands strain under natural conditions produced delayed primary attacks, usually at about 8 months after infection. When infections were induced experimentally, however, the majority (51 out of 87) had early primary attacks (less than 21 days). However, on the basis of reports by Schüffner *et al* (1929) (obviously not their own data), Winckel stated that there are 3, not 2, types of vivax strains which can be separated by virtue of their life pattern; the tropical Chesson and Madagascar strains which have early primary attacks with frequent and early relapses; the temperate zone St. Elizabeth strain which has early primary attacks but late relapses; and the Netherlands temperate zone strain which displays delayed primary attacks followed by frequent relapses.

More recent studies tend to suggest that this classification of tropical versus temperate zone malaria is not all-inclusive. For example, we have studied a Central American (Panama), and certainly "tropical", strain of vivax and observed latent periods of approximately 5 months after exposure to infection or 4 months after treatment of the primary attack. In similar studies involving a Venezuelan strain of *P. vivax,* 5 of 6 volunteers who experienced relapse activity had their first relapse 110 to 335 days after exposure to infection. Only one of the 6 had a relapse as early as 29 days; the latest was at day 609. These results would tend to indicate that long term relapses are not confined to temperate zone strains.

On the basis of life-pattern, Nikolaiev (1949) differentiated 2 subspecies of vivax malaria. One was characterized by short incubation periods of 7 to 23 days (from the southern part of U.S.S.R.), which he designated *P. vivax vivax.* The other subspecies (from northern and central areas of U.S.S.R.), characterized by long incubation periods (from 253 to 381 days), he called *P. vivax hibernans.* These designations failed to receive wide acceptance. Another Russian study in the same vein was that of Tiburskaya (1961) who described a strain isolated in 1953 from a patient who had never left Moscow. The author passed the strain for 5 years via *Anopheles labranchiae atroparvus* mosquitoes and in 103 infections the incubation periods were short (9 to 20 days), but in 13 they were extended (216 to 327 days). These data are somewhat reminiscent of our studies with St. Elizabeth vivax.

Most of the strains of vivax malaria studied to date have short prepatent periods. The greatest or most significant differences appear to lie in their relapse patterns; namely, a very short latent period or a very long latent period, between the primary attack and the first relapse.

We have purposefully omitted details of clinical human malaria because this work is concerned with the biology and parasitology of the primate malarias and, too, such information is well covered in numerous textbooks. We have included, however, the more general and pertinent aspects of human malaria.

Vivax malaria infections are considered to have relatively benign characteristics. Observations of large numbers of vivax infections, allowed to continue until terminated spontaneously, suggest that instances of death due to vivax malaria, in otherwise healthy adults, must indeed be very rare (Kitchen, 1949).

Whorton *et al* (1947) reported that prodromal symptoms before the primary attack in cases of Chesson vivax were almost never observed. Kitchen (1949) stated that prodromal symptoms are usually manifest in persons most susceptible to malaria. In our experience, with several strains of vivax malaria in non-immune volunteers,

prodromal symptoms have not been commonly observed; but, when present, consisted mainly of headache and sometimes generalized malaise.

In non-immune individuals, the onset of the primary attack is usually characterized by a slight, rigorless paroxysm. Kitchen (1949) stated that the course, or sequence of events, of the uninterrupted attack of vivax is characterized by remittent fever, followed by intermittent fever, and then to remission or spontaneous termination of the primary attack. During the period of remittent fever, which lasts generally from 2 to 5 days, the continuous fever curve is characterized by quotidian fever spikes. These daily fever spikes exhibit progressively higher values reaching a maximum shortly after the appearance of intermittent fever. James (1926) reported that fever at the onset of primary attacks of Indian and Madagascar strains may be continuous or remittent over a period ranging from 1 to 3 days and that, if the early fever was intermittent, it was usually quotidian, rarely tertian. Kitchen (1949) emphasizes that this intermittent fever in the completely susceptible person is also quotidian. During the earliest intermittent paroxysms, certain characteristics are conspicuous: 1) the parasite density is mounting; 2) the paroxysms exhibit gradually increasing peak temperatures; 3) the duration of the paroxysm is protracted; and 4) a rigor does not introduce the first few intermittent paroxysms.

Whorton *et al* (1947) reported that in patients infected with Chesson strain vivax, 80 percent had remittent fever at the onset. Of the 20 percent who had intermittent fever at the beginning, 84 percent were quotidian and only 16 percent exhibited tertian fever spikes. The duration of remittent fever in that strain varied from 24 to 184 hours. They suggested that remittent fever, at the onset of the primary attack was probably related directly to irregular segmentation of the parasite. The more typical intermittent febrile paroxysms *are* directly related to the maturation and segmentation of one brood of parasites. During the initial remittent fever, segmentation occurs at irregular intervals (James, 1926; Boyd, 1941).

In our experience, with wholly susceptible volunteers infected with Chesson vivax, the intermittent fever has been tertian as frequently as it has been quotidian. The characteristic of the fever pattern is a reflection of: 1) a single highly synchronous brood of parasites which produces sharp fever spikes, 2) an asynchronous single brood which produces plateau-like fever, and 3) multiple broods which produce daily paroxysms. If the infection is allowed to run, it is not unusual for multiple broods to get-in-step resulting in a tertian fever pattern; likewise, tertian patterns, sometimes, become quotidian for a time, only to revert to tertian again.

In a series of mosquito-induced infections with *P. vivax,* the mean duration of the primary attack was observed to be 19.3 days (Boyd *et al,* 1936a). Paroxysms accompanied by chills are indicative of a more severe paroxysm and usually attended by a greater degree of fever than those without chills. The mean maximum temperature for 654 paroxysms, with chills, was 104.2° F and for 346 paroxysms, without chills, was 102.5° F (Kitchen, 1949). Maximum paroxysmal temperatures are usually attained during the early part of the second week.

Kitchen and Putnam (1946), in a large series, noted that chills introduced up to 71 percent of all paroxysms. Chills were observed to last an average of 50 to 55 minutes. They stated that the temperature at the beginning of the chill was, as a rule, not in the febrile range; i.e., under 100° F and that, as a rule, fever appeared shortly after the onset of the chill, with an average increase of over 4° F (maximum increase was 7° F).

Boyd (1941) stated that chills are not observed at the onset of primary vivax infections and may not be evident for one or even 2 weeks. He reported that chills did occur in their patients during the period of remittent fever but were infrequent (only 26 percent of 144 patients). In a total 158 patients, only 46 percent had chills during any stage of the primary attack, with the first chill being observed as early as the 8th day. However, the incidence of chills might have been higher had primary attacks been allowed to continue.

Coatney and Young (1942) reported on detailed studies of 338 paroxysms in 21 infections

with the St. Elizabeth strain of vivax malaria in Caucasian patients. They observed chills in only 201 (59 percent) of the paroxysms. They suggested that the absence of chills in as many as 41 percent of all paroxysms indicated that the term "chills and fever" does not adequately describe a malarial paroxysm. They found that the average temperature at the onset of each chill was 100.6° F. In addition, the average duration of the chill was 39 minutes; the average temperature rise was 2.3° F; the average time from onset of fever (100° F) to peak fever was 3 hours, 52 minutes; and the average duration of temperature 100° F and above, was 10 hours, 10 minutes. They observed that the average rate of fever rise was 3.3 times faster during the chill (1° F in 17 minutes) than during any other period of fever rise (1° F in 56 minutes). In the chill accompanied paroxysms, fever peaks averaged 0.7° F higher and duration of fever averaged 2 hours shorter than paroxysms without chills. It is generally thought that during this period, the patient is cold because he is shaking; but in truth, he is getting hotter all the time as can be seen in Figure 8. It is well recognized that the rise in a patient's temperature is associated with the maturation of the asexual parasites and that the peak of segmentation precedes the fever peak. It follows then, that the time between fever peaks (paroxysms) is a measure of the length of the asexual cycle which in a one-brooded infection of vivax is said to be 48 hours. However, when precise measurements of periodicity were carried out by Young (1944), it was discovered that the length of the paroxysmal interval for the St. Elizabeth strain was 43 hours, 25 minutes; with a New Hebrides strain the interval was 45 hours, 46 minutes; and with a Baltimore strain the interval was 41 hours, 31 minutes. Also, according to Young (loc. cit.) the fever charts published by Marchiafava and Bignami (1894) showed intervals of less than 48 hours. Kitchen (1949) was concerned with the total paroxysm-picture in contrast to Young's precise measurements and showed that only 28.9 percent were isochronous, 31.6 percent occurred more than 48 hours after the preceding paroxysm, and that 39.6 percent occurred less than 48 hours after the preceding paroxysm. In his experience, paroxysms less than 48 hours apart were more conspicuous during the early primary attack.

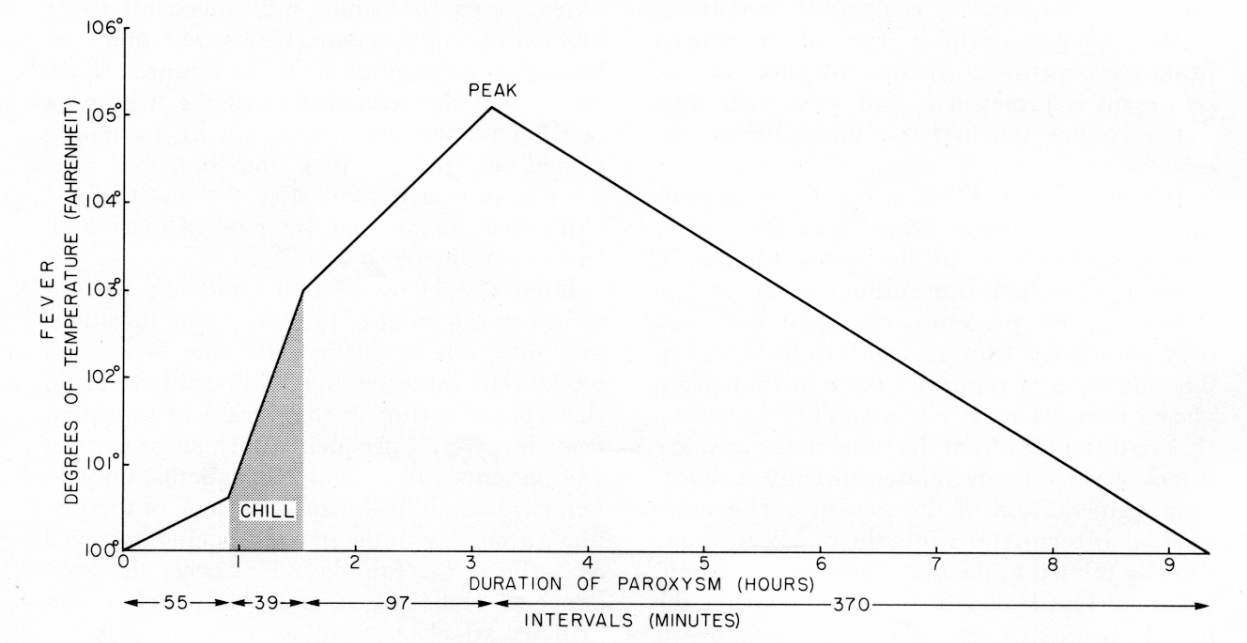

FIGURE 8.—The temperature curve in relation to the chill in 201 paroxysms in *Plasmodium vivax* infections (after Coatney and Young, 1942).

Whorton *et al* (1947) reported the mean duration of fever over 103° F rectally in 235 paroxysms of Chesson vivax was 7.7 hours (only the first 3 paroxysms of the primary attack were measured). The maximum fever observed by them was 108.2° F rectally which occurred on the 10th day of a primary attack.

Fever in the experiences with vivax malaria reported by Kitchen (1949) frequently was found to exceed 106° F; temperatures of 107° F were uncommon. Kitchen (1949) reported an instance of a temperature recording of 107.6° F in a patient who reacted to this high temperature by convulsion--a rare occurrence.

As stated earlier, vivax infections are generally considered to have benign characteristics. In some patients, a pronounced exaggeration of one or more of the usual signs and/or symptoms may be evident and this phenomenon is probably related more to the variability of the host rather than to the parasite. Symptomatology generally includes headache, anorexia, backache, nausea with or without vomiting, myalgia, abdominal pain, and generalized malaise. Hepatomegaly and splenomegaly (up to 7 cm. below the left costal margin) with or without tenderness are not uncommon during the primary attack. Rarely, the spleen may extend into either the lower left or right quadrant. Tenderness is quite variable and not always proportional to the degree of enlargement (Kitchen, 1949).

Whorton *et al* (1947) found the subjective symptoms of Chesson vivax to be similar to those described for other strains. During the initial stage of remittent fever, malaise was almost universal. The other complaints were similar to those mentioned above. Headache was strikingly frequent, often very severe and persistent. They observed that the spleen can become palpable as early as the second day of illness. In most instances, the maximum parasite density is attained between the 7th and 14th days and maximum parasitemia rarely exceeds 50,000 parasites per mm^3 and usually remains below 25,000 per mm^3. Kitchen (1949) reported one vivax infection with a maximum parasite count of 96,000 per mm^3 which was not attended by any alarming symptoms.

Figure 9 shows the minimum and maximum parasitemia curves for 20 infections with the St. Elizabeth strain of *P. vivax* in our Caucasian patients. In addition, there are median parasitemia curves for blood-induced and sporozoite-induced infections. It can be seen that the median peak parasitemia for blood- and sporozoite-induced infections was reached on day 9 and 10 with maximum median counts of 8,365 and 6,775, respectively. After attaining peak parasitemia, the median curves gradually decreased so that by days 20, 30, 40, and 60, the median counts for the sporozoite-induced infections were approximately

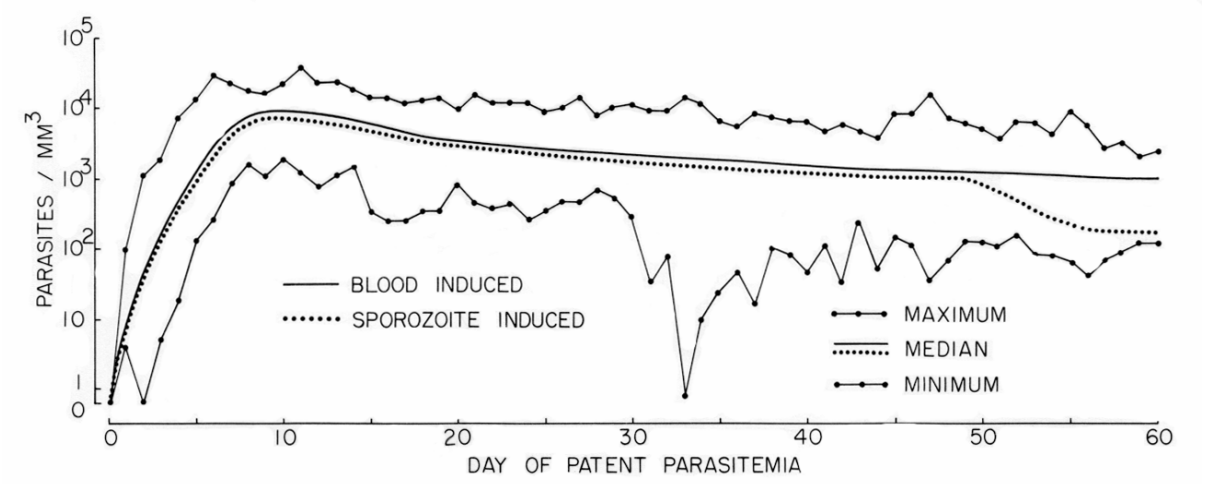

FIGURE 9.—Minimum and maximum parasitemias and median parasitemias curves for blood- and sporozoite-induced infections with St. Elizabeth strain *Plasmodium vivax* in Caucasian patients.

3,000, 2,000, 1,000, and 100 parasites per mm^3, respectively. This difference is evident in the figure at approximately 50 days. The parasitemia in blood-induced infections similarly decreased except that at days 40 and 60 the median counts were approximately 2,000 and 1,000 parasites per mm^3, respectively. The maximum parasite counts for the blood-induced infections were 38,673 on day 11 and 23,232 on day 12 for the sporozoite-induced infections.

Figure 10 shows the parasitemia curves (minimum, median, maximum) for infections in 10 Negro patients with either the Chesson, St. Elizabeth, or Korean strain of vivax malaria. Exposure to infection was by the inoculation of parasitized blood or by sporozoites. It can be seen that the median peak parasitemia (6,850 parasites per mm^3) obtained on day 7 and that the median parasitemia then very slowly but steadily declined. One of the most interesting facts evident in this figure is the maximum parasitemia of 45,844 per mm^3 observed in one patient on day 7. Even more interesting, is the fact that this patient had experienced previous infections with ovale and malariae malaria. Six of the 10 Negro patients infected with vivax malaria had maximum parasitemias greater than 10,000 parasites per mm^3. Three of the 10 Negro patients had experienced previous malaria infections.

An unexplained racial insusceptibility or resistance to infection with many strains of vivax malaria has been repeatedly reported for the Negro. Mayne (1932) first reported that American Negroes were relatively insusceptible to infection with vivax malaria. In 1933, Boyd and Stratman-Thomas also reported Negroes to be generally refractory to vivax malaria. Boyd (1934) showed the Negro was more immune to vivax than falciparum or malariae malaria. Young *et al* (1946, 1955) showed Negroes were more resistant than Caucasians to sporozoite induced vivax malaria. Whorton *et al* (1947a) observed that 6 of 8 Negroes inoculated with heavily parasitized blood failed to develop patent parasitemias. The other 2 showed evidence of partial refractoriness. Young *et al* (1955) reported that only 23 percent of American Negroes developed patent infections with vivax malaria as compared to a 96 percent infection rate in Caucasians. West African Negroes (Liberians) were found to be susceptible to infection with the Madagascar strain of vivax malaria in only 3.3 percent (1 out of 30) of the attempts (Bray, 1958).

Rhesus monkeys and other macaques have not been found susceptible to *P. vivax* either after the intravenous inoculation of heavily parasitized blood or large numbers of sporozoites and malariologists despaired of finding any of the monkeys susceptible to human malaria.

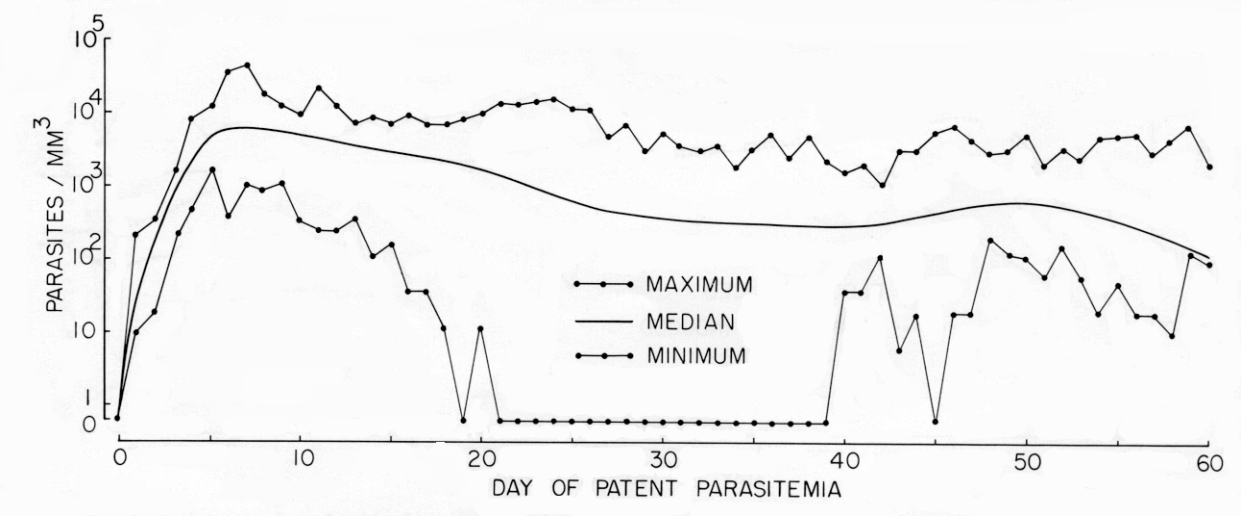

FIGURE 10.—Minimum, median, and maximum parasitemia curves in Negro patients infected with either Chesson, St. Elizabeth, or Korean strain vivax malaria.

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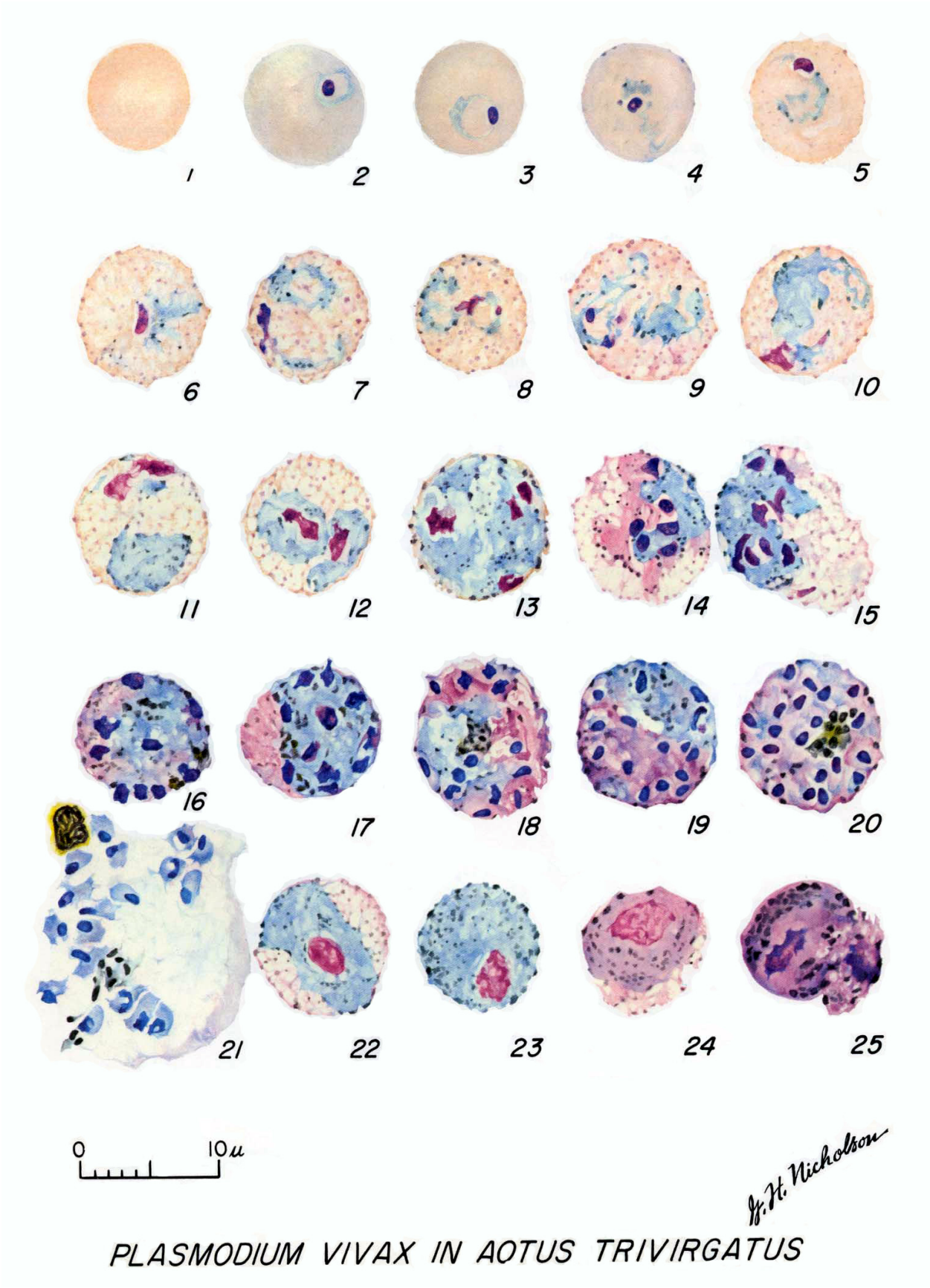

PLASMODIUM VIVAX IN AOTUS TRIVIRGATUS

However, in 1966, Young *et al* and Porter and Young reported that the owl or night monkey, *Aotus trivirgatus,* from Central America was susceptible to infection with *Plasmodium vivax.*

In our studies involving New World monkeys with human malarias, we have been able to establish 7 strains, or isolates, of vivax malaria in owl monkeys. In fact, 6 of the 7 strains were established in intact non-splenectomized animals. (Plate III depicts the appearance of *Plasmodium vivax* in the blood of this animal.) Once detectable parasites were present, the parasitemias rose fairly rapidly (Fig. 11) to a level of approximately 800 per mm^3 by day 11. After a drop in the median parasitemia by day 14, to approximately 100 per mm^3, the parasitemia rose to a level of approximately 1500 per mm^3 by day 18 and slowly declined thereafter. At the end of the 30-day observation period, the median parasite count was approximately 500 per mm^3. Subsequent passage of the infections into splenectomized *A. trivirgatus* monkeys resulted in a more rapid rise in the median parasitemia curve to a maximum level of approximately 10,000 per mm^3 by day 23. At the end of the 30-day observation period, the median parasitemia was approximately 1500 per mm^3.

The parasitemia curve for an *A. trivirgatus* monkey infected with a strain of *P. vivax* from West Pakistan is presented in Figure 12. This animal was splenectomized prior to the intravenous inoculation of parasitized blood. On the 14th day after inoculation, daily feeding of *Anopheles freeborni* mosquitoes was initiated. Even though this was the first day gametocytes were found in the peripheral blood film, they were highly infectious to the mosquitoes. This high level of infection was maintained for the next 9 days followed by a gradual drop in the infection rate until day 32, when no mosquito infections were obtained.

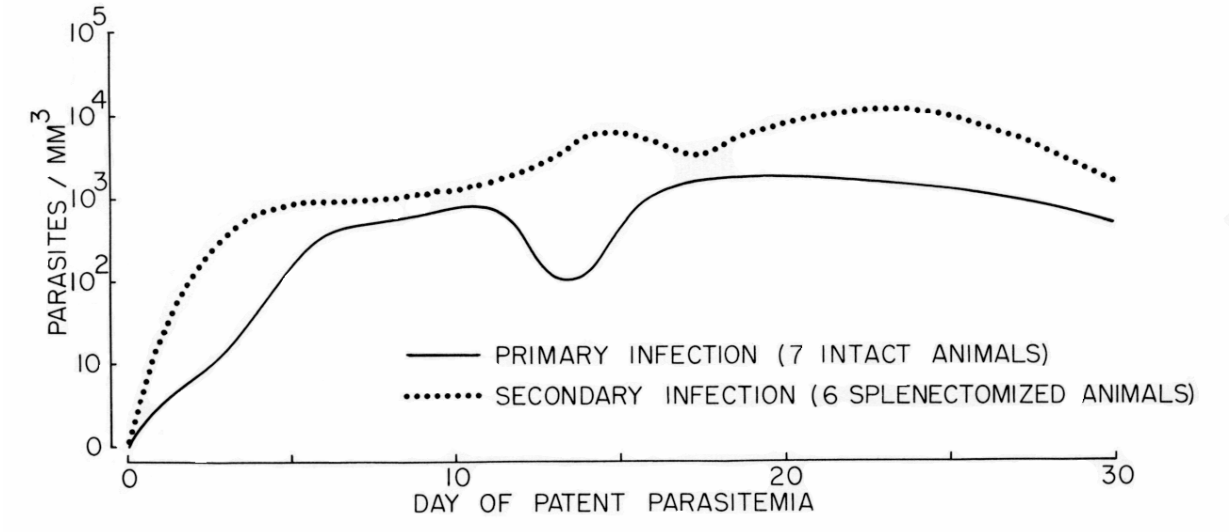

FIGURE 11.—Median parasitemia curves for primary (from man to monkey) and secondary (monkey to man) infections of *Plasmodium vivax* in *Aotus trivirgatus* monkeys.

PLATE III.—*Plasmodium vivax* in the night monkey, *Aotus trivirgatus*.

Fig. 1 Normal red cell.
Figs. 2-5. Young trophozoites.
Figs. 6-9. Growing trophozoites.
Figs. 10, 11. Mature trophozoites.
Figs. 12-17. Developing schizonts.
Figs. 18-21. Nearly mature and mature schizonts.
Figs. 22, 23. Developing and mature macrogametocytes.
Figs. 24, 25. Nearly mature and mature microgametocytes.

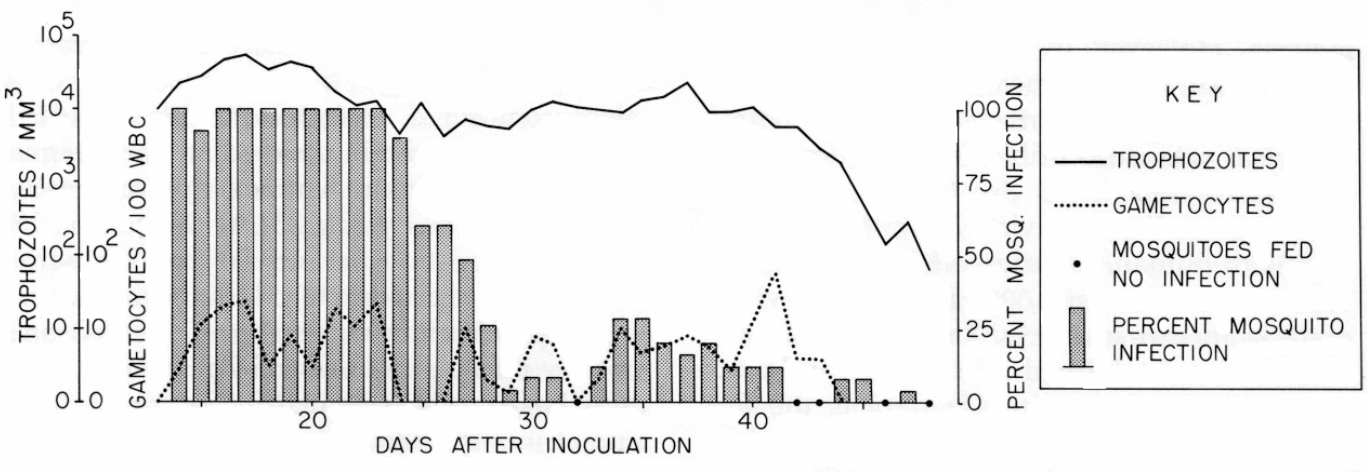

FIGURE 12.—Parasitemia and infectivity rates of *Anopheles freeborni* mosquitoes after feedings on an *Aotus trivirgatus* monkey (AO-91) infected with the West Pakistan strain of *Plasmodium vivax*.

This was followed by a second period of lower-level infectivity of 9 days. The animal died 49 days after inoculation. The fact that an infection of *P. vivax* in the *Aotus trivirgatus* monkey could be infectious to mosquitoes over an extended period of time (in this case, 30 of 35 feeding days), indicates a great potential for their use in experiments necessitating large numbers of infected mosquitoes.

Rodhain (1956) and Bray (1957) have shown that chimpanzees are partially susceptible to *P. vivax,* in that the liver of the chimpanzee would support development of exoerythrocytic parasites but did not support the development of the erythrocytic stages well. However, when the animal, harboring the Madagascar strain, was splenectomized, a high parasitemia appeared in a short time. Prior to splenectomy, no erythrocytic parasites had been observed in smears of the peripheral blood. Subsequently, exposure of 2 splenectomized chimpanzees to infection with this same strain of *P. vivax* quickly resulted in patent infections which developed high parasitemias.

In 1956, Garnham *et al* infected a chimpanzee with fresh blood parasitized by the Madagascar strain of vivax. The parasite did not grow well in the chimpanzee but well enough so that *A. stephensi* mosquitoes could be infected. After a suitable incubation period, the mosquitoes were allowed to bite one of the authors. He became infected. Parasitemia appeared on day 10 and when the patient was taken to the hospital on day 14, his temperature was 41.2° C. The infection was that of a typical vivax showing that the parasite had not been altered by its sojourn in the chimpanzee.

Cadigan *et al* (1968) were able to infect a gibbon with *P. vivax* even though only transient infection obtained.

Host Specificity

Man is apparently the only natural host for *Plasmodium vivax.* Experimental infections have been induced, however, in chimpanzees (Mesnil and Roubaud, 1917, 1920; Garnham *et al,* 1956) and in gibbons, *Hylobates lar,* (Cadigan *et al,* 1968). In 1966, Porter and Young reported the successful infection of owl monkeys, *Aotus trivirgatus,* and Geoffroy's tamarin, *Saguinus geoffroyi,* by the inoculation of parasitized blood from man. In addition, Young *et al* (1966) and Porter and Young (1966) reported the successful transmission of *P. vivax* from the owl monkey to man via the bites of *Anopheles albimanus* mosquitoes. These findings prompted intensive study of *P. vivax* in South American monkeys. Deane *et al* (1966) reported the experimental infection of splenectomized squirrel monkeys, *Saimiri sciureus,* with *P. vivax* and Young and Porter (1969) reported the infection of spider monkeys, *Ateles fusiceps* and *A. geoffroyi,* along with the white-faced monkey, *Cebus capucinus,* with *P. vivax* from *A. trivirgatus* donors.

Baerg *et al* (1969) reported transmission of *P. vivax* from *Aotus trivirgatus* and *Ateles fusiceps*

to *Ateles fusiceps, A. trivirgatus,* and *S. geoffroyi* via the bites of *A. albimanus* mosquitoes, but only with some difficulty. However, Ward *et al* (1969) showed that the Chesson strain of *P. vivax* in the *Aotus trivirgatus* and the chimpanzee could be transmitted with relative ease to *Aotus trivirgatus* and the chimpanzee via the bites of *A. b. balabacensis, A. stephensi,* and *A. quadrimaculatus* mosquitoes.

The almost worldwide distribution of *P. vivax* is indicative of the large number of mosquitoes capable of transmitting the parasite. Of the 5 species of *Anopheles* routinely used in our laboratory (Table 2), *A. stephensi* was the most susceptible to infection with it, followed by *A. b. balabacensis, A. freeborni, A. maculatus,* and, finally, *A. quadrimaculatus.*

In this and subsequent chapters, the relative susceptibility of a species of *Anopheles* to infection with a particular species of *Plasmodium* is based on the determination of the average number of oocysts per 100 guts (Gut Infection Index) for a standard mosquito, either *A. freeborni, A. b. balabacensis,* or *A. maculatus,* fed simultaneously with the species being compared. The Gut Infection Index ratios are determined by the relationship of the GII of the standard mosquito to that of the test mosquito; the GII of the standard mosquito is then given an arbitrary rating of 100. In Table 2, *A. freeborni* is given the arbitrary rating of 100. If in a simultaneous feeding of this species and *A. stephensi,* the standard had 10 oocysts per gut, the *A. stephensi* would have had 13.19 oocysts per gut. By this method it is possible to determine the relative susceptibility of all the mosquitoes to each other by their relationship to the standard.

Immunity and Antigenic Relationships

In 1924, Yorke and Macfie demonstrated the existence of homologous strain immunity against *Plasmodium vivax* by showing that after the development of acquired immunity individuals were refractory to superinfection by the same strain. Homologous strain immunity against vivax malaria has since been confirmed repeatedly by Boyd and Stratman-Thomas (1933a), Boyd *et al* (1936), Boyd and Matthews (1939), Boyd (1942), Boyd and Kitchen (1943,1946), and Jeffery (1956). These investigators were able to show that the development of the immunity obtained in subjects infected either by sporozoites or by parasitized blood. The phenomenon was manifest in the form of decreased parasite density, shorter duration of patent parasitemia, and/or decreased clinical manifestations.

Boyd and Kitchen (1943, 1946) stated that abundant evidence, gathered by themselves and others, existed to show that persons convalescing

TABLE 2.--Comparative infectivity of *Plasmodium vivax* to *Anopheles freeborni, A. stephensi, A. b. balabacensis, A. maculatus,* and *A. quadrimaculatus.*

Mosq. species comparison	Number tests	Number of mosquitoes		Percent infection		GII** ratio
		Standard	Other	Standard	Other	
F-1						100
F-1 : St-1	6	38	44	86.8	84.1	131.9
F-1 : Bal	11	88	61	68.2	82.0	109.3
F-1 : Mac	24	271	272	47.6	42.3	47.0
F-1 : Q-1	9	82	108	86.6	66.7	41.2

* F-1 = *Anopheles freeborni;* St-1 = *A. stephensi;* Bal = *A. b. balabacensis;* Mac = *A. maculatus;* Q-1 = *A. quadrimaculatus.*

** GII = Gut Infection Index = average number of oocysts per 100 guts; the GII ratio is the relationship of the GII of *A. freeborni* to another species where the GII *of A. freeborni* = 100.

from an infection with vivax malaria had acquired potent immunity to the strain of parasite which produced their attack. While recovery from an attack of vivax malaria is indicative of acquired immunity to the strain which excited the attack, the same individual may be expected to exhibit a subclinical parasitemia subsequent to a reinoculation with the same strain of parasite. Boyd and Kitchen (1946) showed that the level of immunity in convalescing malarial infections could be increased by a series of subsequent reinoculations with the same strain of parasite. Oftentimes, a level of immunity (hyperimmunity) could be attained where the individual was able not only to withstand, but also to promptly destroy, large inocula of parasites, indeed, many million times greater than the minimal number required to infect a non-immune individual.

Boyd, Stratman-Thomas, and Kitchen (1936) reported that an effective homologous immunity to the McCoy strain of vivax malaria could persist for more than 3 years. Boyd and Matthews (1939) reported that 2 patients still showed signs or evidence of immunity 7 years after their primary experience with the homologous strain. The signs of immunity were diminished parasitemia, increased clinical tolerance, and accelerated activation of immune mechanisms.

Boyd and Kitchen (1943) made 2 attempts to transfer the hyperimmune state passively through the transfusion of large volumes of blood. Both attempts failed to prevent infection or modify the course of infection in susceptible persons.

Jeffery (1956) reported that patients reexposed to homologous strain infection of Chesson vivax, by bites of infected mosquitoes, were found to usually experience infections with a shorter and milder course, and, after treatment, a single relapse ensued which was usually asymptomatic.

Nicole and Steel (1926) reported that immunity may also exist between heterologous strains of vivax malaria. Boyd (1942) confirmed this observation with American strains of vivax malaria. Ciuca *et al* (1937) reported a relative cross-immunity in vivax malaria between imported strains, and strains indigenous to Roumania.

Boyd *et al* (1934), based on their studies with induced vivax malaria, reported that previous infections with malaria do reduce the severity of subsequent infections. They concluded that superinfections with heterologous strains of vivax malaria result in clinical attacks of milder intensity than the original ones.

Boyd *et al* (1939) reported that an absence of cross-immunity between species of malaria *(P. vivax* and *P. falciparum)* was observed whether superinfection occurred during the incubation period, during the acute primary attack, or shortly after recovery from the acute attack.

Whorton *et al* (1947a) in their studies with the Chesson strain of vivax malaria discussed innate or natural immunity as well as acquired immunity. They reported that of 8 Negroes inoculated intravenously with blood parasites of the Chesson strain, all were partially or completely refractory to infection. Four of the patients developed neither patent parasitemia nor fever. This phenomenon has been reported by many investigators. For example, Young *et al* (1955) in their studies of induced human malaria in patients reported that Negroes generally demonstrated a refractoriness to infections with many domestic and foreign strains of vivax malaria under conditions in which Caucasian patients were wholly susceptible.

Tobie and Coatney (1961) were the first to report that antisera to *P. vivax* and to *P. cynomolgi* would cross-react with the heterologous antigens. Voller (1962) showed that such cross-reactions would also occur between *P. vivax* and *P. bastianellii* (= B strain *P. cynomolgi), P. gonderi,* and *P. osmaniae* (= OS strain *P. inui).* Further studies by Tobie *et al* (1962) indicated that although considerable cross-reaction was obtained, the maximum antibody titer tended to be the homologous antigen. Diggs and Sadun (1965) studied the cross-reactivity of sera from volunteers infected with *P. falciparum* and others infected with *P. vivax,* using the IFA technique, and found that sera from patients with *P. falciparum* had geometrical mean reciprocal titers of 1:28.2 against the homologous antigen in contrast to 1:6.3 against the *P. vivax* antigen. In a reverse study, sera from patients with *P. vivax* infections had homologous geometrical mean titers of 1:17.2 and heterologous mean titers of 1:9.3.

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(NS) = Not seen.